UNIVERSITY OF PENNSYLVANIA

WATER EXCHANGE THROUGH THE EGG MEMBRANES OF FUNDULUS

ELIZABETH M. YAGLE

A THESIS

IN ZOOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

PHILADELPHIA

1930

WATER EXCHANGE THROUGH THE EGG MEMBRANES OF FUNDULUS

A THESIS

IN ZOOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF PENNSYLVANIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

ELIZABETH M. YAGLE

Reprint from

Protoplasma, Vol. IX, No. 2

PHILADELPHIA

1930

PRINTED IN GERMANY

R. UNIV
BIBLIOTHEEK
LEIDEN

PERMEABILITY OF EGG MEMBRANES

WATER EXCHANGE THROUGH THE EGG MEMBRANES OF FUNDULUS

ELIZABETH M. YAGLE

Zoology Laboratory, University of Pennsylvania and
the Biological Laboratory, Cold Spring Harbor

With 14 Text-figures

Received for publication, July 17, 1929

The passage of water and other substances through membranes has been a subject of extensive study by MICHAELIS (24), LOEB (16), NORTHROP (26) and others. These investigators have worked largely with membranes prepared of collodion or of collodion in combination with other substances such as gelatin, albumin and lecithin.

LOEB (8) in 1912 suggested a method of studying the rate of water exchange through living membranes by determining the effects of various salt solutions on the floating time of *Fundulus* eggs when the latter are immersed in these solutions. He found that these eggs, which normally have a specific gravity higher than that of sea water, floated in 12/8 M NaCl but not in 11/8 M NaCl; in 3 M NaCl they floated for not more than three hours, then sank to the bottom of the test tube; in 10/8 M $CaCl_2$ or higher concentrations they floated at first but sank within a half-hour. In a mixture of 50 C.C. 3 M NaCl and .2 C.C. 10/8 M $CaCl_2$ eggs floated for 3 days or longer, while in a mixture of 50 C.C. $2^1/_2$ M NaCl, 1 C.C. $2^1/_2$ M KCl and 0.75 C.C. $2^1/_2$ M $CaCl_2$ some eggs floated as long as 10 days. LOEB considered the sinking of the egg due to an equalization of the specific gravities of the egg and the solution probably by means of the exosmosis of water from the egg, water exchange being controlled by the permeability of the egg membrane (the chorion). Permeability was increased by solutions of single salts but decreased by solutions of certain mixtures of these

salts, indicating, as pointed out by LOEB, an antagonistic action of the cations of the salts. Mixtures of NaCl, KCl and $CaCl_2$ in the proportions found in sea water were designated balanced salt solutions; solutions of single salts or of combinations of salts that showed no antagonistic action were called unbalanced solutions.

A study of LOEB's work suggested the possibility of using his method as a means of determining something further of the nature of the water exchange through the egg membranes. Experiments were accordingly carried out by the methods described below.

METHODS

Eggs of *Fundulus heteroclitus* were procured by stripping female fish into a bowl of sea water; sperm were obtained by removing and teasing testes in a small quantity of sea water. Several drops of sperm suspension were added to the eggs which were then kept at room temperature, 22° to 26° C. The sea water was changed daily. In experiments unfertilized eggs were used as well as those 1, 2, 3, 4 and 9 days after fertilization. Eggs of these ages seemed to represent distinct stages in development. In the 1-day embryo there are visible the embryonic shield and the first line indicating the position of the body. In the 2-day embryo the body is well formed and the optic lobes are prominent. In 3 days the heart has developed and is beating but circulation is not as yet established. At 4 days, the circulation is well established and the chromatophores are visible. By the ninth day the embryo is well developed, the fins are capable of movement and the chromatophores are arranged in a definite pattern. Few marked morphological changes take place after the ninth day. The fish hatch in 12 to 14 days.

Solutions of c.p NaCl, KCl and $CaCl_2$ in distilled water were used in molar concentrations ranging from 3/8 to 14/8 molar. Solutions of mixtures of these salts were made in the proportions suggested by LOEB (8), as optimum antagonistic proportions, namely Na: K = 17:1; Na: Ca = 50:1; K: Ca = 300:1; Na: K: Ca = 100:2.2:1.5.

Changes in the hydrogen-ion concentrations of the solutions were made by adding varying amounts of HCl or NaOH, pH values being determined colorimetrically. By preliminary experiments it was determined that the cation Na of the NaOH solution used was not present in sufficient quantity to affect the reactions of the KCl and $CaCl_2$ solutions to which it was added.

Chemically clean test-tubes 13.5 cm. by 2 cm. with a capacity of about 34 c.c. were placed in a rack and filled with the various solutions to a height of 13 cm.

Individual eggs were removed from the finger-bowls with a medicine dropper, drained on filter paper and laid carefully at the top of the solution just *under* the surface film in order to avoid floating of the egg *on* the surface film. In preliminary experiments the eggs were rinsed in distilled water for a few seconds before being placed in the solution. As there seemed to be no difference in reaction between these and the unrinsed eggs, the rinsing process was omitted in subsequent experiments.

The time of floating at the top of the solution and the time of sinking through a column of 13 cm. were measured by means of a stop-watch.

All experiments were carried out at room temperatures of 22° to 26° C., temperature during any one experiment, however, remaining constant to within $\pm 1.0°$ C.

RESULTS OF EXPERIMENTS

THE TIME AND RATE OF SINKING OF EGGS

By means of the technic described above the sinking time of *Fundulus* eggs of different ages was determined. Figures 1, 2 and 3 show the time required for eggs to sink in solutions of NaCl, KCl and $CaCl_2$ of various molar concentrations. From an inspection of these curves it is evident that there is a different sinking time for eggs of different ages in each of the solutions used. Since the anion Cl is common to all, the differences noted in their effects must be due to the cations Na, K and Ca. The order for the time of sinking is $Na < K < Ca$ or, conversely, the order for the rate of sinking is $Na > K > Ca$.

It may be thought that since the specific gravity of the solution varies with its molar concentration, the rate of sinking of the egg is directly dependent on the specific gravity of the solution. It can be shown, however, that this is not necessarily the case. If composite curves of the time of sinking in solutions of various molar concentration (Fig. 4) be compared with curves indicating the specific gravities of these solutions (Fig. 5) it will readily be seen that while the specific gravity of the solution increases at a constant rate, the increase in time of sinking of the egg is not constant. The rate of sinking,

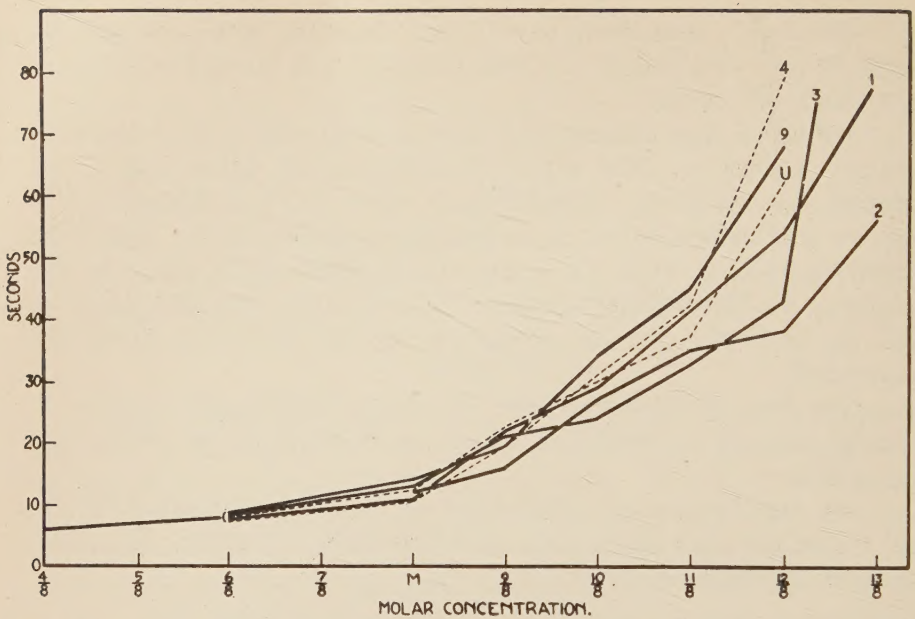

Fig. 1. Shows the sinking time of *Fundulus* eggs in NaCl solutions. 1, 2, 3, 4, 9 indicate the ages of the eggs in days after fertilization; U indicates unfertilized eggs.

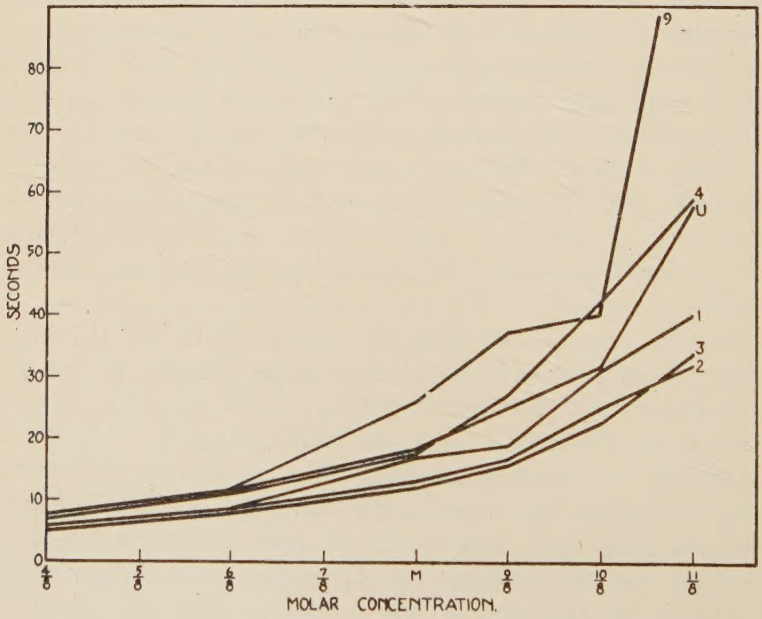

Fig. 2. Shows the sinking time of *Fundulus* eggs in KCl solutions. 1, 2, 3, 4, 9 indicate the ages of the eggs in days after fertilization; U indicates unfertilized eggs.

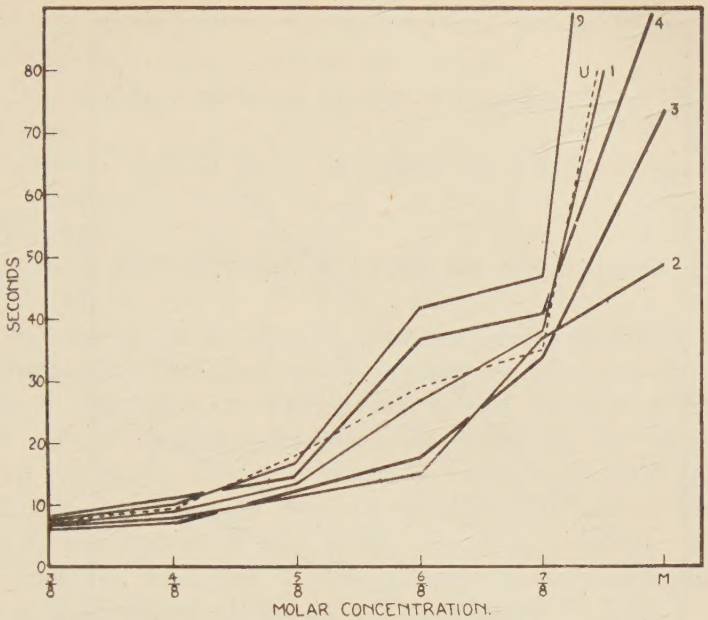

Fig. 3. Shows the sinking time of *Fundulus* eggs in $CaCl_2$ solutions. 1, 2, 3, 4, 9 indicate the ages of the eggs in days after fertilization; U indicates unfertilized eggs.

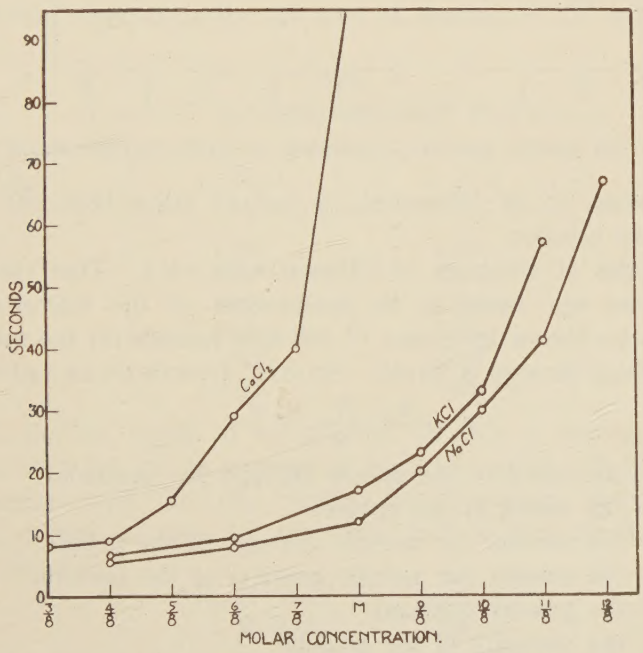

Fig. 4. Shows composite curves of the sinking time of *Fundulus* eggs of different ages in NaCl, KCl and $CaCl_2$ solutions.

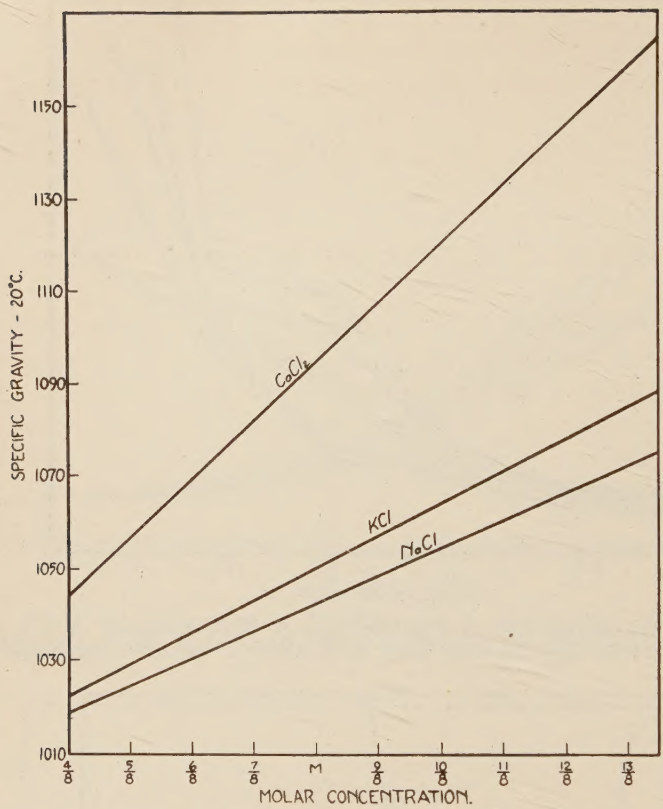

Fig. 5. Shows the specific gravities of solutions of NaCl, KCl and $CaCl_2$ at 20° C.

therefore, seems to be influenced by factors other than the specific gravity of the solution.

Viscosities of solutions of different salts vary. That the rate of sinking of the egg seems to be independent of the viscosity of the solution can be shown by means of STOKES' formula for the velocity of a sphere falling through a liquid. STOKES' formula is as follow:

$$V = \frac{2\,g\,(S—S_1)\,r^2}{9\,u}$$

V = the speed of the sphere through the medium.
r = the radius of the sphere.
S = the density (or specific gravity) of the sphere.
S_1 = the density (or specific gravity) of the medium.
g = the gravity constant.
u = the viscosity of the medium.

V can be determined by dividing the distance of the fall 13 cm. by the time in seconds required for sinking.

The average diameter of an egg of *Fundulus* is 0.2 cm.; therefore r is 0.1 cm.

S_1 for N NaCl at 20° C. is 1.0402; for N KCl 1.0464; for N $CaCl_2$ 1.0448.

$g = 980$

u for N NaCl at 20° C. is 1.1069; for N KCl 0.9955; for N $CaCl_2$ 1.31.

Differences in the specific gravities of the solutions are in this case negligible. Therefore, in the formula we have only two variables, namely V, the speed of sinking and u, the viscosity of the solution. If the rate of sinking were solely dependent on the viscosity of the solution, the ratio of V to u should be constant for each solution and should therefore represent only one variable. The value of the unknown factor S, the density of the sphere, should be constant. If we solve for S, however, we obtain a different value in each solution and at each stage of development of the egg. This is shown in Table I. Since we assume that the initial densities of eggs of the various age groups are constant, these results seem to show that the viscosity of the solution is not solely responsible for the rate of sinking of the egg.

Table I

VALUES OF S IN STOKES' FORMULA

Egg	N. NaCl	N. KCl	N. $CaCl_2$
Unfert.	1.595	1.376	1.91
1 day	1.64	1.395	1.945
2 day	1.595	1.508	2.08
3 day	1.548	1.538	2.181
4 day	1.686	1.395	1.775
9 day	1.511	1.278	1.824

As another means of determining the rate of sinking of the egg, the time required for its passage through the upper half of the solution was compared with the time required for its passage through the lower half. The ratio of time for the upper half to time for the lower half was as follows:

In M NaCl	1.11 : 1
M KCl	1.52 : 1
6/8 M $CaCl_2$	1.33 : 1.

By using the formula for uniformly accelerated motion $s = {}^1/_2\, a\, t^2$ (where s = space; a = acceleration; t = time) we can calculate the acceleration in falling and from this determine the time which should be required for falling through the upper half and through the lower half of the solution. By calculation the ratio of time for the upper half to time for the lower half is 2.5 : 1 for all solutions.

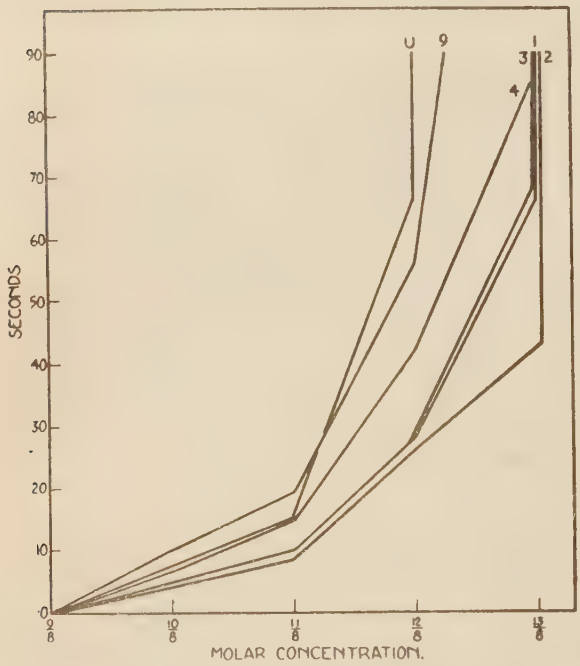

Fig. 6. Shows the floating time of *Fundulus* eggs in NaCl solutions. 1, 2, 3, 4, 9 indicate the ages of the eggs in days after fertilization; U indicates unfertilized eggs.

This shows that in solutions of all three salts the acceleration is less than the calculated value. As the solution is homogeneous throughout the tube the change in rate seems to indicate a change in the specific gravity of the egg, as a result, probably, of a change in permeability of the egg membrane. If the egg remained unchanged throughout its fall, the acceleration would be the same as calculated. If the specific gravity of the egg were increasing, the acceleration would be greater than calculated. It would seem, therefore, that in this case the specific gravity of the egg is decreasing. This indicates an increase in permeability of the egg membrane as a result of which water may be entering the egg or salts may be passing out to lower the specific gravity of the egg.

These experiments on the sinking of the egg of *Fundulus* indicate that each of the salts used has a distinct effect on the rate of sinking and therefore on the permeability of the egg membrane. The effect seems to be due to the cations of the salts. Also a definite difference is shown in the reactions of eggs of different stages of development. As this is considered a point of importance it will be discussed more fully in another section of this paper.

THE TIME AND RATE OF DELAY IN SINKING (FLOATING) OF EGGS

In the preceding experiments there was a period of delay before sinking in some of the solutions, namely those of higher molar concentration than the solutions in which the eggs sank at once. The time of delay in sinking (floating) was determined as well as the time of sinking. Figures 6, 7 and 8 show the curves for the time of delay in

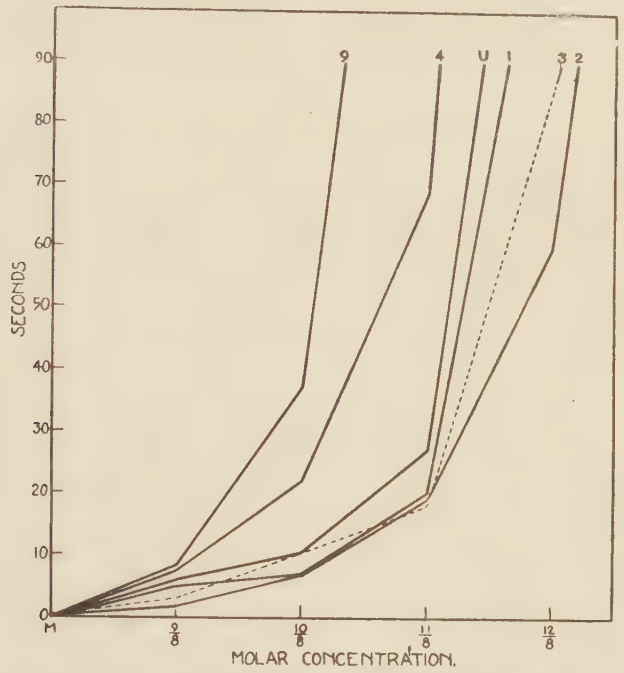

Fig. 7. Shows the floating time of *Fundulus* eggs in KCl solutions. 1, 2, 3, 4, 9 indicate the ages of the eggs in days after fertilization; U indicates unfertilized eggs.

sinking in solutions of NaCl, KCl and $CaCl_2$ of various molar concentrations. Here again the anion Cl is common to all three solutions; therefore the effect of the salts must be due to the cations Na, K and Ca. The effect of these cations on the time of floating is similar to their effect on the time of sinking, consequently the order for the time of delay in sinking is $Na > K < Ca$ and, conversely, for the rate, $Na > K > Ca$.

It is a well-known fact that under normal conditions a solid will sink in a liquid only when it is of a higher specific gravity than the

liquid. One would therefore expect an egg of *Fundulus* to sink only in those solutions which are of a lower specific gravity than that of the egg. This would hold good if the egg were an impermeable solid. The egg, however, is not an impermeable solid but is surrounded by a membrane, the chorion, through which water and probably salts can pass. The specific gravity of the egg changes and an egg which at first floats will sink after a time because of an increased specific gravity due to the passing out of water or the entrance of salts. LOEB (8) has shown that in unbalanced salt solutions the permeability of the chorion becomes much greater than in balanced solutions; consequently the passage of water and salts is facilitated. Salts probably diffuse through the membrane more slowly than water. In the present case we are dealing with unbalanced solutions, that is, solutions of single salts. In such solutions the egg membrane is doubtless permeable to water and salts but in the short time interval represented in these experiments probably only the passage of water takes place. Increase of specific gravity of the egg then seems to be due to exosmosis of water through the egg membrane.

Fig. 8. Shows the floating time of *Fundulus* eggs in $CaCl_2$ solutions. 1, 2, 3, 4, 9 indicate the ages of the eggs in days after fertilization; U indicates unfertilized eggs.

It can be shown that the rate of water exchange, on which the floating of the egg probably depends, is not wholly dependent on the osmotic pressure of the solution. Composite curves of the time of floating in solutions of NaCl, KCl and $CaCl_2$ (Fig. 9) are of a different type from the osmotic pressure curves for the same solutions (Fig. 10). Osmotic pressure, as calculated from dissociation constants of the salts in solutions of various concentrations, increases at a constant rate; the increase in time of floating is not constant.

In Figure 9 the curves given are for iso-osmotic solutions of NaCl, KCl, and $CaCl_2$. These curves show that the time and, therefore, the rate of floating is different in various salt solutions of equal osmotic pressure.

Since the floating of the egg is not entirely dependent on the osmotic pressure of the solution, it must be influenced by some other

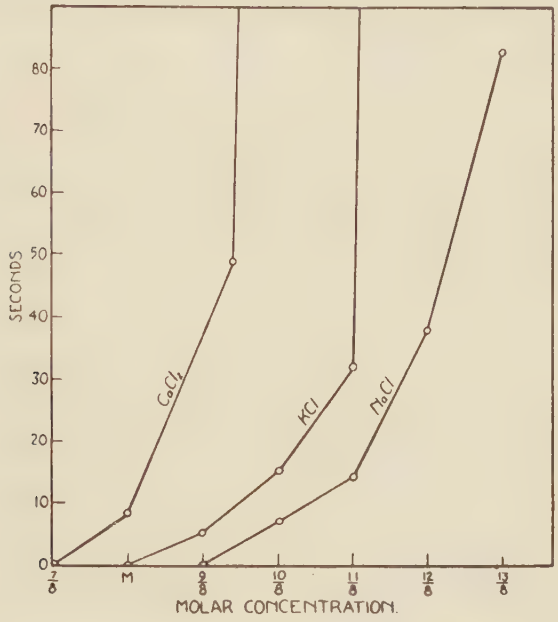

Fig. 9. Shows composite curves of the floating time of *Fundulus* eggs of different ages in NaCl, KCl and $CaCl_2$ solutions.

factor. This factor is probably the specific effect of each of the different salts used, or rather of the cations of these salts Na, K and Ca. The salt effect seems to result in a change in the permeability of the egg membrane whereby the rate of water exchange is increased. The order of the cations in increasing permeability is Na > K > Ca.

In the floating experiments as well as in the sinking experiments differences in reaction of eggs of various ages are brought out (Figs. 6, 7, 8).

THE EFFECT OF FERTILIZATION ON THE RATE OF SINKING AND FLOATING OF EGGS

A point of some interest is the difference in the sinking and floating reactions of *Fundulus* eggs in different stages of development.

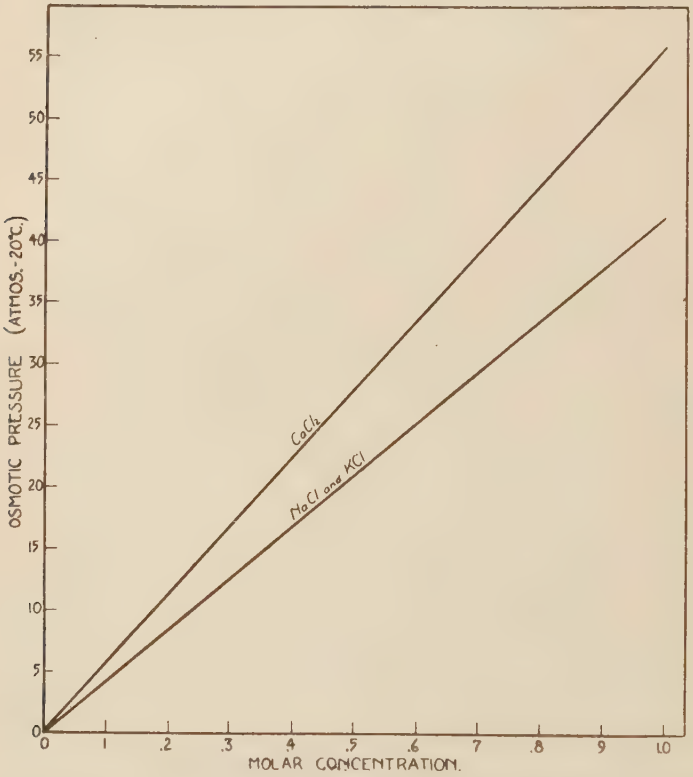

Fig. 10. Shows the osmotic pressures of solutions of NaCl, KCl and $CaCl_2$ at 20° C.

A study of Figures 1, 2 and 3, shows that the time and, therefore, the rate of sinking varies with the stage of development of the egg. In all experiments the 1, 2 and 3 day eggs sank more quickly than the unfertilized, 4, and 9 day eggs. Likewise Figures 6, 7 and 8 show that the time and rate of floating vary with the age of the egg. The 1, 2 and 3 day eggs floated for a shorter time than the unfertilized, 4 and 9 day eggs.

These different rates of sinking and floating indicate a difference in permeability and therefore a definite physiological change in the

membranes of the egg at different stages of development. Various investigators have reported an increase of permeability after fertilization. McClendon (21) in 1910 noted by means of conductivity methods that the permeability of sea urchin eggs increased at the beginning of development. In 1913 the same investigator (22) reported that unbalanced salts increased the permeability of the fertilized egg of *Fundulus* to electrolytes; in 1914 (23) he showed that fertilization increased the permeability of frogs' eggs so that electrolytes diffused out. Lyon and Schackell (20) in 1910, working with sea urchin eggs, found that fertilization increased permeability to methylene blue and dahlia and to oxygen and the pigment of the eggs. Gray (2) in 1913 noted that there was an increase in the conductivity of the sea urchin egg after fertilization but that conductivity returned toward that of the unfertilized egg after 10 to 15 minutes. This second change was temporary and small as compared with the first original increase. Conductivity always remained decidedly greater in fertilized than in unfertilized eggs. The experiments of Lillie (3, 4) in 1917 on the swelling of the sea urchin egg showed that after fertilization or artificial activation swelling was four times as great as in the unfertilized egg. This remained constant at least until the second clearage. Loeb used fertilized *Fundulus* eggs in many of his experiments but made no special point of the time after fertilization or of the stage of development other that to note that the eggs were "newly fertilized" or that they contained embryos in which the hearts were beating. Sumwalt (27) in reporting her investigation in potential differences across the chorion of the *Fundulus* egg states, "The properties of the chorion do not appear to vary greatly with the age of the egg, except for a slight decrease in elasticity with time. Fertilized eggs of any convenient age could therefore be used". Most of the eggs employed in her experiments were between 5 and 9 days old. Other workers using fertilized and unfertilized eggs have made no mention of different reactions at various stages of development.

The reactions obtained with *Fundulus* eggs indicate the advisability of noting the exact stage of development when fertilized eggs are employed in experimental procedure. Differences in reaction at different developmental stages seem to be due to physiological changes in the egg membranes. These changes closely parallel morphological changes in development. Permeability increases up to the time when circulation is established then decreases again until it reaches approximately the same degree as that of the unfertilized egg.

THE EFFECT OF THE HYDROGEN-ION CONCENTRATION ON THE RATE OF SINKING AND FLOATING OF EGGS

In many physiological processes the hydrogen-ion concentration of the medium is a significant factor. For this reason the effect of changes in hydrogen-ion concentration on the sinking and floating of *Fundulus* eggs was studied. Experiments were carried out in the same way as

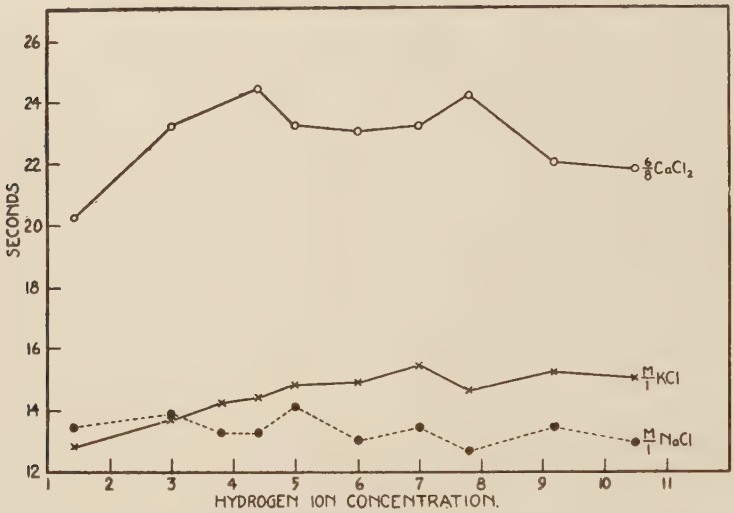

Fig. 11. Shows the effect of the hydrogen-ion concentration (expressed as pH) of solutions of Na Cl, K Cl and $Ca Cl_2$ on the sinking time of *Fundulus* eggs of 4 days' development.

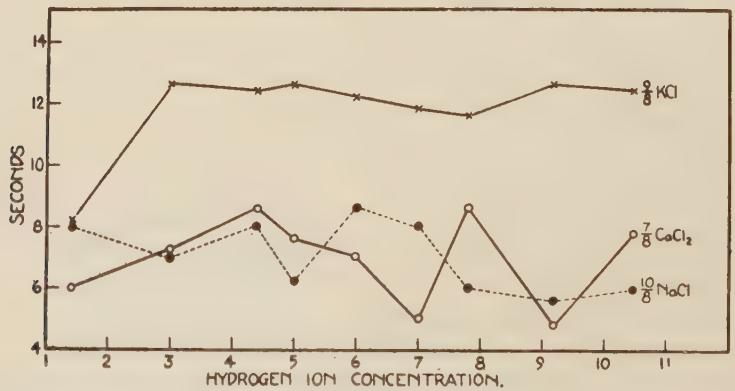

Fig. 12. Shows the effect of the hydrogen-ion concentration (expressed as pH) of solutions of Na Cl, K Cl and $Ca Cl_2$ on the floating time of *Fundulus* eggs of 4 days' development.

the preceding ones except that the salts were used in constant molar concentrations and pH values of the solutions were varied. As relatively similar reactions were obtained with eggs in different states of development only the curves for the 4-day eggs will be shown. Figure 11 shows the effect of the hydrogen-ion concentrations (expressed as pH) of NaCl, KCl and $CaCl_2$ solutions on the sinking time of the egg; Figure 12, the effect on the time of floating.

Variations in the time and hence in the rate of sinking and of floating of the egg are irregular and inconsistent and are found to be no greater than the standard deviation in time in each individual solution. The effect of the hydrogen-ion concentration seems to be of little significance in the sinking and floating reactions of *Fundulus* eggs in the various solutions used. We may conclude, therefore, that the hydrogen-ion concentration probably does not influence the permeability of the egg membrane, at least within the time limits of exposure used in the present tests. This is in accordance with the conclusions of LUCKE and MC CUTCHEON (19) that the hydrogen-ion concentration of the surrounding solution has no effect on the swelling of *Arbacia* eggs.

THE EFFECT OF MIXTURES OF SALTS ON THE RATE OF SINKING AND FLOATING OF EGGS

Many of LOEB's investigations on *Fundulus* eggs dealt with the effects of mixtures of salts and the antagonistic action of different ions (5—15). In these investigations LOEB showed that an increased floating time for the egg in mixtures of NaCl, KCl and $CaCl_2$ was due to a decreased permeability of the egg membrane to water, caused by the antagonistic action of K and C on Na.

It was thought of interest to test further the effects of mixtures of salts on the rate of sinking and floating of *Fundulus* eggs, solutions of NaCl + KCl (17 : 1), NaCl + $CaCl_2$ (50 : 1) and NaCl + $CaCl_2$ (100 : 2.2 : 1.5) were compared with pure NaCl solutions.

Figure 13 shows the effect of mixtures of these salts of various concentrations on the sinking time of *Fundulus* eggs. Figure 14 shows the effect on the time of floating. In both cases curves for 4-day eggs are shown as typical reactions.

An Analysis of Figure 13 shows that the time of sinking is approximately the same in pure NaCl solution and in mixtures of NaCl with KCl and $CaCl_2$. No antagonistic effect is evident, indicating that

antagonistic action, which would decrease the permeability of the egg membrane, does not occur in the short time interval required for sinking. Hence the rate of sinking is unchanged.

Figures 14 shows that the time of floating in mixtures of NaCl and KCl is approximately the same as in pure NaCl solution. In mixtures of NaCl and $CaCl_2$ and in mixtures of NaCl, KCl and $CaCl_2$ there is a slight decrease in the time of floating, indicating an increase in the permeability of the egg membrane. This is the reverse of the

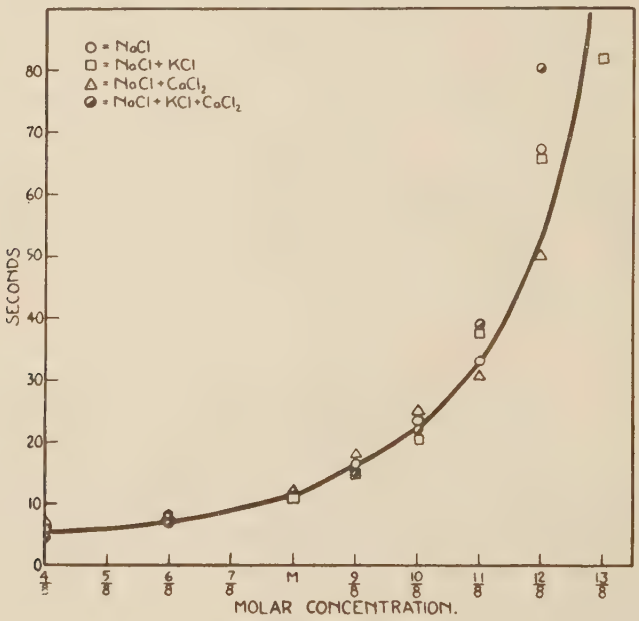

Fig. 13. Shows the effect of mixtures of salts on the sinking time of *Fundulus* eggs of 4 days' development.

Table II

Solution	Time of Floating in	
	14/8 M	2 M
NaCl	1—2 hr.	2—3 hr.
NaCl + KCl	2—3 hr.	2—3 hr.
NaCl + $CaCl_2$ . . .	3—4 hr.	2 days
NaCl + KCl + $CaCl_2$.	1—2 days	5—6 days

decrease in permeability shown in mixtures of the same salts in higher molar concentrations.

The decrease in permeability of the egg membrane with the resulting increase of floating time of the egg can best be shown by means of a table. The above results are in accordance with those of LOEB (8). No marked antagonistic action is shown with mixtures of NaCl and KCl. A decided antagonism, however, is shown with NaCl and $CaCl_2$ and a very marked antagonism with $NaCl + KCl + CaCl_2$. This antagonism renders the egg membrane less permeable to the passage of water and salts and thus prevents a change in specific gravity and subsequent sinking of the egg. Mixtures of NaCl, KCl and $CaCl_2$ are most effective in decreasing permeability.

Antagonistic action of the salts must be due to their cations since the anion Cl is common to all the salts used. LOEB (5) in 1900 showed that NaCl was poisonous to marine animals and that the poisonous effect was due to the Na ions and could be counteracted by Ca or K ions. Later (6) he showed that fertilized eggs of *Fundulus* could

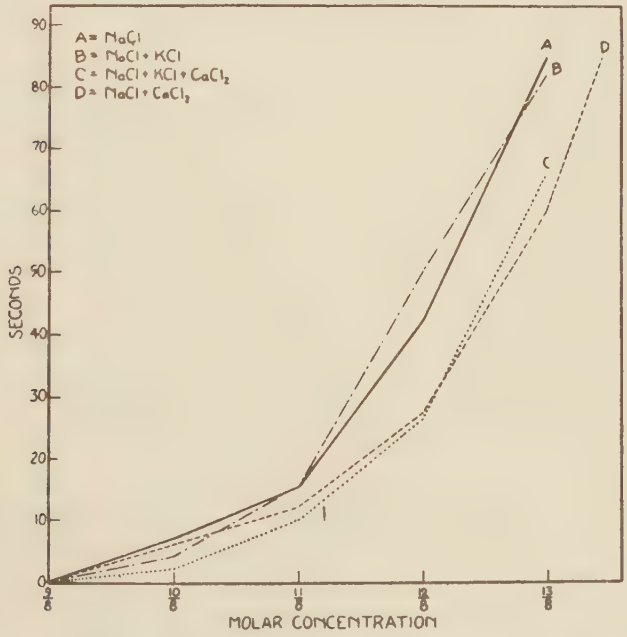

Fig. 14. Shows the effect of mixtures of salts on the floating time of *Fundulus* eggs of 4 days' development.

develope in sea water or distilled water but would die quickly in NaCl of the same concentration as that in sea water. The effect of NaCl could be overcome by the addition of certain other salts, particularly those with bivalent and trivalent cations. LOEB (7) explained this antagonistic action as being due to a modification of the egg membrane by the combined action of two salts. He considered the modification a tanning of the surface film of the egg membrane due to a change in the physical character of the albumin salts according to the nature of the cations or anions with which they were in contact. He also concluded that salts (7) surrounding the membrane enter the egg slowly, the speed of entrance probably being least with the combination NaCl, KCl and $CaCl_2$.

THE EFFECT OF THE CHORION AND THE VITELLINE MEMBRANE ON THE RATE OF WATER EXCHANGE

Surrounding the *Fundulus* egg there are two membranes, an inner delicate vitelline membrane and an outer chorion, which is tough and turgid and much thicker than the vitelline membrane. Between the two is a fluid, the perivitelline, or subchorionic, fluid.

The question arises as to the respective roles played by these two membranes in the water and salt exchange of the egg. Embryos removed from the chorion were subjected to sinking and floating tests in various salt solutions and their reactions compared with those of eggs of the same age with chorions intact. The technic used in removing the chorion was that described by Dr. J. S. NICHOLAS (25) of Yale University. (The writer is greatly indebted to Dr. NICHOLAS for dissecting out most of the embryos used in this experiment.) Eggs of about 8 days' development were used, since the embryos in these were fairly well developed and yet unable to swim.

The time of sinking in M NaCl and M KCl and in 6/8 M $CaCl_2$ solutions was determined. Likewise, the time of floating was tested in 10/8 M NaCl, 10/8 M KCl and 7/8 M $CaCl_2$ and in solution of mixtures of these salts.

In the M NaCl, M KCl and 6/8 M $CaCl_2$ solutions both the intact egg and the egg without the chorion began to sink at once. In all these solutions, however, the intact egg sank more quickly than the egg without the chorion, indicating that the weight of the former was sligthly greater than that of the latter. As the sinking time of both was short probably no significant changes occurred in the membranes during the time interval involved.

Table III

Solution	Floating Time	
	Intact Egg	Egg without Chorion
10/8 M NaCl	16 sec.	2 sec.
10/8 M KCl	65 sec.	53 min.
7/8 M $CaCl_2$	11 sec.	25 min.
10/8 M NaCl + 10/8 M KCl	15 sec.	10 min.—2 hr.
10/8 M NaCl + 7/8 M $CaCl_2$	20 sec.	6 sec.
10/8 M KCl + 7/8 M $CaCl_2$	20 sec.	28 min.
10/8 M NaCl + 10/8 M KCl + 7/8 M $CaCl_2$	12 sec.	10 sec.

The floating time of intact eggs and eggs without the chorions in various salt solutions is shown in Table III.

Certain distinct differences in the reactions of the intact egg and the egg without the chorion are evident. If variations in the time of floating were due only to differences in the specific gravities of the eggs compared, one would expect the floating reactions of these eggs to be relatively the same in all solutions. This, however, is not the case.

In pure NaCl solutions and in mixtures of NaCl + $CaCl_2$ and NaCl + KCl + $CaCl_2$ the intact egg floated a little longer than the egg without the chorion but both began to sink in a relatively short time. It seems, therefore, both in the case of the chorion and of the vitelline membrane that the action of NaCl is purely osmotic and that sinking of the intact egg and the egg without the chorion is due to exosmosis of water through their respective membranes. The vitelline membrane seems to be more freely permeable to water than the chorion, hence the rate of water exchange is faster than through the chorion and the egg with the chorion removed sinks sooner than the intact egg. If the rate of water exchange through the vitelline membrane were not faster than through the chorion the intact egg would sink sooner than the egg without the chorion, since, as shown above, the intact egg is the heavier. NaCl probably does not pass through either membrane in the time involved. Antagonistic action of Ca or of Ca + K is of little significance in either case.

On the other hand, in KCl, $CaCl_2$, KCl + $CaCl_2$ and NaCl + KCl, the egg without the chorion floated a much longer time than the intact egg. This may indicate (1) that the rate of water exchange is much slower through the vitelline membrane than through the chorion, or (2) that

the chorion is not permeable to salts in a short period of time while the vitelline membrane is freely permeable. Since the results of the action of NaCl seem to indicate that the vitelline membrane is more freely permeable to water than is the chorion, it is hardly probable that in KCl and $CaCl_2$ the permeability ratio would be reversed. The second assumption seems more reasonable. In this case the chorion is not rendered permeable to salts in the short period of time involved but it is permeable to water. The specific gravity of the intact egg is increased by exosmosis of water through the chorion and the egg sinks. If the vitelline membrane is permeable to KCl and $CaCl_2$ these salts will enter and equalize the pressure of the solutions on the inside and outside of the membrane. No osmotic pressure difference will be set up and the embryo will float until the effect of KCl or $CaCl_2$ or both together becomes injurious to the embryo. In mixtures of NaCl + KCl the effect of the action seems to be that of KCl rather than NaCl.

These conclusions seem to be in accordance with those of BODINE (1) in the action of salts on the intact egg of *Fundulus* and on the egg without the chorion. He found that the action of NaCl was osmotic, that is, NaCl spent all of its time effect on the chorion and did not penetrate either the chorion or the vitelline membrane. KCl and $CaCl_2$, however, penetrated both the chorion and the vitelline membrane.

As the experiments on the effect of the chorion and the vitelline membrane on the rate of water exchange were only preliminary experiments and were conducted with small numbers of eggs, it seems hardly justifiable to draw definite conclusions from them.

DISCUSSION

Rates of sinking and floating in various salt solutions have been established for *Fundulus* eggs in different stages of development. We have assumed that these rates are due to differences in rates of water exchange through the chorion of the egg, this exchange resulting from osmotic pressure differences on the chorion. Permeability of the chorion to water was increased or decreased by various salts, or by the cations of these salts, their order in increasing permeability being Na > K > Ca.

Another interpretation of these results is possible, namely that the change of specific gravity of the egg is not essentially due to exosmosis of water through the chorion but is due to the diffusion of salts through the chorion into the egg. That electrolytes do diffuse through the membranes has been shown by LOEB (5—17) in his numerous

investigations on salts, acids, and alkalies. LOEB and WASTENEYS (18) by cryoscopic methods have shown that the osmotic pressure of the *Fundulus* egg does not change with the osmotic pressure of the surrounding solution if the eggs are kept in distilled water, normal sea water or concentrated or diluted sea water. These results would indicate that salts do not enter the egg. This holds good, however, only for balanced solutions such as sea water and combinations of Na, K and Ca salts in proper antagonistic proportions. Unbalanced salts diffuse through the chorion.

Other investigators also have shown that salts penetrate the egg membranes. THORNER (29), by determining the time of recovery of heart-beat in embryos in which the heart-beat had been completely blocked by KCl, found that the time of recovery was directly proportional to the concentration of KCl and to the time of immersion in KCl. Recovery of heart-beat was an indication of the diffusing of KCl out of the egg. That this really did occur was checked by keeping eggs with blocked heart-beat in distilled water and testing the water at intervals for the presence of electrolytes. By means of conductivity measurements it was shown that there was a daily increase in the amount of electrolytes in the water indicating that some electrolytic substance was extruded through or at least given off from the membranes of the egg. BODINE (1) showed that KCl and $CaCl_2$ seem to penetrate the egg membranes at all stages of development while NaCl seems to exert most of its effect on the chorion of young eggs but diffuses through the membranes of other eggs. SUMWALT (27) has shown by measuring the potential differences across the chorion of the *Fundulus* egg that in neutral solutions the chorion is more permeable to cations than to anions but as the hydrogen-ion concentration of the solution is increased the ratio of anion to cation permeability is increased and finally inverted.

SVETLOV (28) recently determined the osmotic pressure of the perivitelline fluid and of the yolk of trout eggs by means of microcryoscopic methods. He found that the osmotic pressure of the perivitelline fluid is always the same as that of the solution surrounding the egg, indicating that the chorion is freely permeable to salts. That the vitelline membrane is not permeable was shown by the fact that the osmotic pressure of the yolk was always found to be constant. It is questionable whether the reactions of eggs of a fresh water animal are comparable with those of a salt water animal.

In none of these investigations has the time of initial entrance of the salts into the egg been determined, but consensus of opinion seems to be that the salts enter slowly and a period of some minutes or even hours may be required before the salts reach the inside of the chorion.

In the sinking and floating experiments presented in this paper, the time involved has ranged from a few seconds to about two minutes, hence the time interval is probably shorter than that in which salts penetrate the chorion of the egg.

We can therefore conclude that differences in the rates of sinking and floating of *Fundulus* eggs in various salt solutions are due to the exosmosis of water from the egg rather than to the entrance of salts. The method presented may be useful in determining the rate of water exchange through living cell membranes.

SUMMARY

1. A method is described for measuring the rate of water exchange through living membranes.

2. By this method definite rates of water exchange for *Fundulus* eggs of different ages were established in solutions of NaCl, KCl and $CaCl_2$ of various molar concentrations. The effect of these salts in increasing permeability seems to be due to their cations, the order of effectiveness being $Na > K > Ca$.

3. Marked physiological differences in the membranes of eggs in various developmental stages are indicated.

4. The effect of the hydrogen-ion concentration of the solution on the rates of water exchange is of little significance.

5. Antagonistic effects of mixtures of salts are not evident in the time interval involved in the experiments.

6. The rôles of the chorion and the vitelline membrane in the water and salt exchange of the egg of *Fundulus* are discussed.

I am deeply indebted to Dr. J. H. BODINE of the University of Pennsylvania for the suggestion of this problem and for his continued assistance and encouragement throughout the investigation. My thanks are due also to Dr. R. H. HARRIS, Director of The Biological Laboratory, Cold Spring Harbor, for providing research facilities.

LITERATURE CITED

1. Bodine, J. H. 1928. Action of salts on *Fundulus* egg. Biol. Bull., **54**, 396.
2. Gray, J. 1913. Quoted by Lillie, R. S. Am. J. Physiol., **45**, 406.
3. Lillie, R. S. 1916. Increase of permeability to water following normal and artificial activation in sea urchin eggs. Am. J. Physiol., **40**, 249.
4. — 1918. The increase of permeability to water in fertilized sea urchin eggs and the influence of cyanide and anaesthetics upon this change. Am. J. Physiol., **45**, 406.
5. Loeb, J. 1900. The poisonous character of a pure Na Cl solution. Am. J. Physiol., **3**, 327.
6. — 1905. Weitere Bemerkungen zur Theorie der antagonistischen Salzwirkungen. Arch. ges. Physiol., **107**, 252.
7. — 1911. Über den Mechanismus der antagonistischen Salzwirkungen. Biochem. Zeitschr., **36**, 275.
8. — 1912. Antagonistic action of electrolytes and permeability of the cell membrane. Science, **36**, 637.
9. — 1915. On the role of electrolytes in the diffusion of acid into the egg of *Fundulus*. J. Biol. Chem., **23**, 139.
10. — 1915. The mechanism of antagonistic salt action. Proc. Nat. Acad. Sc., **1**, 473.
11. — 1916. Antagonistic salt action as a diffusion phenomenon. Science, **44**, 574.
12. — 1916. The mechanism of diffusion of electrolytes through animal membranes. Proc. Nat. Acad. Sc., **2**, 511.
13. — 1916. The mechanism of the diffusion of electrolytes through the membranes of living cells. (a) I. The necessity of the general salt effect upon the membrane as a prerequisite for the diffusion. J. Biol. Chem., **27**, 339; (b) II. The diffusion of K Cl out of the egg of *Fundulus* and the relative efficiency of different ions for the salt effect. Ibid., 353; (c) III. The analogy of the mechanism of the diffusion for acids and potassium salts. Ibid., 363.
14. — 1917. V. The additive effect of salt and base and the antagonistic effect of salt and acid. J. Biol. Chem., **32**, 147.
15. — 1922. Sodium chloride and selective diffusion in living organisms. J. Gen. Physiol., **5**, 231.
16. — 1923. Influence of salts on the rate of diffusion of acid through collodion membranes. J. Gen. Physiol., **5**, 255.
17. — and McCattell, McK. 1915. The influence of electrolytes upon the diffusion of potassium out of the cell and into the cell. J. Biol. Chem., **23**, 41.
18. — and Wasteneys, H. 1915. Note on the apparent change of osmotic pressure of cell contents with the osmotic pressure of the surrounding solution. J. Biol. Chem., **23**, 157.
19. Lucke, B. and McCutcheon, M. 1925. The effect of hydrogen-ion concentration on swelling of cells. J. Gen. Physiol., **9**, 709.
20. Lyon, E. P. and Schackell, L. F. 1910. Increased permeability of sea urchin eggs following fertilization. Science, **32**, 249.

21. McClendon, J. F. 1910. Electrolytic experiments showing increase in permeability of the egg to ions at the beginning of development. Science, **32**, 122.
22. — 1913. The relation between abnormal permeability and abnormal development of *Fundulus* eggs. Science, **38**, 280.
23. — 1914. The preservation of the life of the frog's egg and the initiation of development by increase in permeability. Am. J. Physiol., **35**, 163.
24. Michaelis, L. and Weech, A. A. 1929. Studies on permeability of membranes. VIII. The behavior of the dried collodion membrane toward bivalent cations. J. Gen. Physiol., **12**, 487.
25. Nicholas, J. S. 1927. The application of experimental methods to the study of developing *Fundulus* embryos. Proc. Nat. Acad. Sc., **13**, 695.
26. Northrop, J. H. 1928. The permeability of the dry collodion membranes. J. Gen. Physiol., **11**, 233.
27. Sumwalt, M. 1929. Potential differences across the chorion of the *Fundulus* egg. Biol. Bull., **56**, 193.
28. Svetlov, P. 1929. Entwicklungsphysiologische Beobachtungen an Forelleneiern. Arch. f. Entwicklungsmechanik, **114**, 771.
29. Thorner, M. H. 1929. Recovery of the heart-beat of *Fundulus* embryo after stoppage by potassium chloride. Biol. Bull., **56**, 157.